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INHIBITION OF RETROVIRUS INFECTION

This invention relates to the field of the treatment of retroviral infections and, more particularly, to the treatment of human immunodeficiency virus (HIV) infection and associated disease, including acquired immune deficiency syndrome (AIDS).

Cross Reference to Related Applications

This application is a continuation-in-part of co-pending United States Patent Application No. 07/943,369, filed September 9, 1992.

Background of the Invention

Retroviral agents have been implicated in a number of diseases, including cancer, autoimmune disease and AIDS. Human immunodeficiency virus (HIV) infection causes chronic progressive depletion of $CD4^+$ T lymphocytes ($CD4^+$ cells) and infection of macrophages, resulting in acquired immune deficiency syndrome. Currently zidovudine (AZT), an analogue of thymidine, is the primary anti-viral drug used in the treatment of HIV infection, although two other agents with a similar mechanism of action, dideoxyinosine (ddI) and dideoxycytosine (ddC), are also used. Colley, T.P. et al., New Engl. J. Med. (1990) 322:1340-45; Fischl, M.A., et al., New Engl. J. Med. (1987) 317:185-91. These agents are effective in inhibiting viral replication, and can stabilize the $CD4^+$ cell levels, but they are unable to eliminate one of the major viral reservoirs, HIV infected macrophages. Gartner, S., et al., Science (1986) 233:215-19. Severe toxicity, particularly involving HIV host bone marrow is also associated with higher doses of AZT treatment, and the beneficial effects of the drug in AIDS patients diminishes after prolonged therapy; HIV strains resistant to AZT also have been observed in treated patients. These findings have prompted the search for alternative drugs for

the treatment of HIV infection, particularly agents with a different mechanism of action.

Human immunodeficiency virus type 1 (HIV-1), a retrovirus, is the etiologic cause of AIDS. The HIV-1 envelope glycoprotein, gp120, specifically binds to the CD4 receptor on T lymphocytes and on monocytes and macrophages. Although infection of T lymphocytes requires cellular proliferation and DNA synthesis, productive infection of monocytes can occur independently of cellular DNA synthesis (Weinberg, J.B., et al,. (1991) J. Exp. Med. 174:1477-82). When HIV-1 infects activated $CD4^+$ lymphocytes, it is lethal, but infected monocytes are relatively resistant to destruction by the virus. Consequently, these cells, once infected with HIV-1, serve as long-lived reservoirs of the virus. Not only are these cells a source of replicating virus, but their virally-mediated dysfunction may contribute to increased susceptibility to opportunistic infections that are the hallmark of AIDS.

Because monocyte-macrophages serve as reservoirs for HIV-1, selective targeting of this population, in addition to T lymphocytes, warrants further consideration (Finberg, R.W., et al., Science 252:1703-05, 1991. Early reports from Fox's group (JADA 118:709-711, 1989) indicated that a component of human saliva blocks HIV replication. More recently, Hattori (FEBS Lett. 248:48-52, 1989) showed that an inhibitor of tryptase (a trypsin-like enzyme) can inhibit syncytia formation of T-cells induced by HIV.

In exploring various potential modulators of HIV-1 infection, we have recently identified an endogenous source of inhibitory activity which retards HIV-1 infection and/or replication.

The factor responsible for the antiviral activity is serine leukocyte protease inhibitor (SLPI). SLPI is a potent inhibitor of human leukocyte elastase, chymotrypsin, cathepsin G, and of human trypsin, and has been purified from parotid secretions (Thompson, R.C. and K. Ohlsson, (1986) Proc. Natl. Acad. Sci. USA,

83:6692-96; and U.S. Patent No. 4,760,130, both of which are incorporated herein by reference). SLPI is now available through production by recombinant DNA techniques; U.S. patent application No. 07/712,354, filed June 7, 1991, PCT application No. WO86/03519, filed December 4, 1985, and European patent application 85 905 953.7, filed December 4, 1985, each of which are incorporated herein by reference).

The ability of SLPI and/or its derivatives and analogs to block HIV-1 infection and/or replication can provide the basis for therapeutic intervention in HIV-1 infection.

Summary of the Invention

The present invention provides novel methods for preventing or treating retroviral infections of mammalian cells, particularly preventing infection of human cells with human immunodeficiency virus (HIV) and associated diseases, including acquired immune deficiency syndrome (AIDS).

Included within the scope of this invention are pharmaceutical compositions for treating retroviral infections, particularly HIV infections in a human, comprising serine leukocyte protease inhibitor (SLPI), or an analog or derivative thereof, and a pharmaceutically acceptable carrier.

The invention also includes a method for treating HIV infections in a human cell comprising administering thereto an effective amount of SLPI or an analog or derivative thereof.

Brief Description of the Figures

Figure 1. SLPI blocks HIV replication in monocytes in a dose-dependent manner. Elutriated human monocytes were plated and exposed to HIV ± SLPI for one hour at 37°C, washed, and incubated at 37°C, drawing off supernatants and adding fresh medium every four days. The EC_{50} for this experiment was <0.1 μg/ml (8.5 nM) with complete inhibition at 10 μg/ml (850 nM).

Figure 2. The SLPI inhibitory effect is long-lasting. At the 18-day time point, HIV is still 90% inhibited.

Detailed Description of the Invention

The present invention provides methods for preventing retrovirus, particularly HIV infection of mammalian cells, particularly human cells, and associated diseases, including acquired immune deficiency syndrome (AIDS).

The term "pharmaceutically acceptable carrier" as used herein means a non-toxic, generally inert vehicle for the active ingredient, which does not adversely affect the ingredient or the patient to whom the formulation is administered.

The term "effective amount" as used herein means a pre-determined amount of SLPI, or an analog or derivative thereof, sufficient to be effective against HIV in vivo.

According to the present invention, retroviral infections are treated by administering anti-retroviral agents in doses sufficient to diminish the effects of such infection. Retroviral infections are implicated in a number of diseases, including but not limited to cancer, autoimmune disease, and acquired immune deficiency syndrome. Human immunodeficiency virus infection is of particular interest according to the present invention.

A variety of anti-retroviral agents are known in the art. Most of these inhibit the activity of retroviral reverse transcriptase and include zidovudine (AZT), an analogue of thymidine, dideoxyinosine (ddI), and dideoxycytosine (ddC). Zidovudine is the primary anti-viral drug used in the treatment of HIV infection. Anti-retroviral agents are generally efficacious in a dose ranging from about 50 mg/day to about 1000 mg/day, more particularly from about 100 mg/day to about 500 mg/day, and in the case of zidovudine, specifically about 300 mg/day to about 500 mg/day. These agents are generally administered in oral formulations.

The protease inhibitors used in this invention can be prepared by means well known to those skilled in the art (see, e.g., U.S. Patent No. 4,760,130; European patent application 85 905 953.7, PCT application WO86/03519, and U.S. patent application 07/712,354, supra).

The present invention relates to protease inhibitors which have been isolated in a purified form. Preferably, the serine protease inhibitors of the present invention are single-polypeptide-chain proteins which are substantially homologous to, and most preferably biologically equivalent to, the native serine protease inhibitor isolated from human parotid secretions. The native serine protease inhibitor is also referred to as the native parotid inhibitor. By "biologically equivalent" as used throughout the specification and claims, is meant that the compositions are capable of inhibiting the monocyte-derived or T-cell derived protease that is inhibited by SLPI, but not necessarily to the same degree. By "derivatives" as used throughout the ensuing specification and claims, is meant a degree of amino acid homology to the native parotid inhibitor, preferably in excess of 40%, most preferably in excess of 50%, with a particularly preferred group of proteins being in excess of 60% homologous with the native parotid inhibitor. The percentage homology, as above described, is calculated as the percentage of the components found in the smaller of the two sequences that may also be found in the larger of the two sequences, a component being understood as a sequence of four, contiguous amino acids.

One useful SLPI derivative is CLPI, a truncated SLPI molecule having only the last 60 amino acids of the native parotid inhibitor. These 60 amino acids are:

Leu Asp Pro Val Asp Thr Pro Asn Pro Thr Arg Arg Lys

Pro Gly Lys Cys Pro Val Thr Tyr Gly Gln Cys Leu Met

Leu Asn Pro Pro Asn Phe Cys Glu Met Asp Gly Gln Cys

Lys Arg Asp Leu Lys Cys Cys Met Gly Met Cys Gly Lys Ser Cys Val Ser Pro Val Lys Ala. (SEQ. ID. NO.: 1)

The following nucleotide sequence has been used to encode the above 60 amino acid molecule:

CTG GAT CCT GTT GAC ACC CCA ACA CCA ACA AGG AGG AAG
CCT GGG AAG TGC CCA GTG ACT TAT GGC CAA TGT TTG ATG
CCT AAC CCC CCC AAT TTC TGT GAG ATG GAT GGC CAG TGC
AAG CGT GAC TTG AAG TGT TGC ATG GGC ATG TGT GGG AAA
TCC TGC GTT TCC CCT GTG AAA GCT. (SEQ. ID. NO.: 2)

CLPI has been constructed by deleting from the SLPI gene the signal sequence and the nucleotides corresponding to the first 47 amino acids of the mature SLPI protein as described in U.S. patent application 07/712,354. CLPI can also be made by the method of Example 8 described in both PCT application WO86/03519 and European patent application 85 905 953.7. Although Example 8 in these two applications recites a method of making SLPI, this method can also be used to make CLPI. CLPI can be used to generate antibodies useful in purifying SLPI. Antibodies can be produced, for example, by the methods discussed in E. Harlow & D. Lane, Antibodies: A Laboratory Manual, pp. 92-114 (Cold Springs Harbor Laboratory, 1988).

By "analogs" as used herein, is meant any compound, including, for example, small organic compounds, that are functionally biologically equivalent to SLPI in inhibiting HIV infection. Such derivatives and analogs can be isolated by means well known to those skilled in the art, including using monocyte cells or T cells to screen for compounds that prevent SLPI from binding thereto. Analogs may also include specific SLPI muteins that have at least equivalent, and in some cases, greater activity than the native protein. Particularly useful SLPI muteins include substitution of the following amino acids at the residue position enumerated: Gly 20, Gly 72, Val 72, and Phe 72.

CLPI muteins are also within the scope of the invention. CLPI muteins which correspond to the SLPI muteins Gly 72, Val 72, and Phe 72 are herein referred to as Gly 25, Val 25, and Phe 25. Some contemplated CLPI muteins have the following amino acid sequence:

Leu Asp Pro Val Asp Thr Pro Asn Pro Thr Arg Arg Lys
Pro Gly Lys Cys Pro Val Thr Tyr Gly Gln Cys R_8 R_3
R_9 Asn Pro Pro Asn Phe Cys Glu R_4 Asp Gly Gln Cys
Lys Arg Asp Leu Lys Cys Cys R_5 Gly R_6 Cys Gly Lys
Ser Cys Val Ser Pro Val Lys R_7

wherein R7 is alanine, and R3, R4, R5, R6, and R8 are the same or different amino acids and one or more of R3, R4, R5, R6, and R8 may be methionine, valine, alanine, phenylalanine, tyrosine, tryptophan, lysine, glycine, or arginine. (SEQ. ID. NO.: 3) Analogs also include, for example, PEGylated forms of SLPI or CLPI which may have improved therapeutic characteristics over the native SLPI protein. Muteins which may be suitable for PEGylation include those having a cysteine residue at positions 13, 23, 52, 58, 68, and/or 75 of SLPI and at the corresponding sites 5, 11, 21, and 28 in CLPI. Preparation of cysteine muteins for PEGylation is described in PCT application WO 92/16221, filed March 13, 1992, which is specifically incorporated herein by reference. A useful step in mutein production can include a refolding step in which cysteine is added to the solution containing the protein. The cysteine can aid in refolding and can bond to the substituted free cysteine in the mutein. One may also isolate from monocytes or T cells the SLPI inhibitable protein (SIP) from human monocyte cells or human T cells using standard biochemical techniques well known to those skilled in the art and purify proteins having proteolytic activity which is inhibited by SLPI. After purifying the protein (and, if necessary, sequencing it, cloning its gene, and expressing it in host cells, i.e., recombinantly producing SIP), one can screen for inhibitors of SIP

by means well known to those skilled in the art. Alternatively, one can determine its structure and design inhibitors therefrom, also by means well known to those skilled in the art.

When SLPI, or an analog or derivative thereof (collectively, the "compounds"), is used to combat HIV infections in a mammal, the compound can be administered orally, parenterally, or locally, in a vehicle comprising one or more pharmaceutically acceptable carriers, the proportion of which is determined by the solubility and chemical nature of the compound, chosen route of administration and standard biological practice.

Pharmaceutical compositions containing the compounds of the present invention can be prepared. These compounds can be in a pharmaceutically-acceptable carrier to form the pharmaceutical compositions of the present invention. The term "pharmaceutically acceptable carrier" as used herein means a non-toxic, generally inert vehicle for the active ingredient, which does not adversely affect the ingredient or the patient to whom the composition is administered. Suitable vehicles or carriers can be found in standard pharmaceutical texts, for example, in Remington's Pharmaceutical Sciences, 16th ed., Mack Publishing Co., Easton, PA (1980), incorporated herein by reference. Such carriers include, for example, aqueous solutions such as bicarbonate buffers, phosphate buffers, Ringer's solution and physiological saline. In addition, the carrier can contain other pharmaceutically-acceptable excipients for modifying or maintaining the pH, osmolarity, viscosity, clarity, color, sterility, stability, rate of dissolution, or odor of the formulation.

The pharmaceutical compositions can be prepared by methods known in the art, including, by way of an example, the simple mixing of reagents. Those skilled in the art will know that the choice of the pharmaceutical carrier and the appropriate preparation of the composition depend on the intended use and mode of administration.

In one embodiment, it is envisioned that the compound and pharmaceutically acceptable carrier constitute a physiologically-compatible, slow-release formulation. The primary solvent in such a carrier can be either aqueous or non-aqueous in nature. In addition, the carrier can contain other pharmacologically-acceptable excipients for modifying or maintaining the pH, osmolarity, viscosity, clarity, color, sterility, stability, rate of dissolution, or odor of the formulation. Similarly, the carrier can contain still other pharmacologically-acceptable excipients for modifying or maintaining the stability, rate of dissolution, release, or absorption of the compound. Such excipients are those substances usually and customarily employed to formulate dosages for oral, parenteral or local administration in either unit dose or multi-dose form.

Once the pharmaceutical composition has been formulated, it can be stored in sterile vials as a solution, suspension, gel, emulsion, solid, or dehydrated or lyophilized powder. Such formulations can be stored either in a ready to use form or requiring reconstitution immediately prior to administration.

The manner of administering the formulations containing the compounds for systemic delivery can be via subcutaneous, intramuscular, intravenous, oral, intranasal, or vaginal or rectal suppository. Administration of the formulations containing the compounds for local delivery includes via intraarticular, intratracheal, or instillation or inhalations to the respiratory tract. Local administration via vaginal or rectal suppository or topical application is also contemplated. In addition it may be desirable to administer the compounds to specified portions of the alimentary canal either by oral administration of the compounds in an appropriate formulation or device.

For oral administration, the compound can be formulated in unit dosage forms such as capsules or tablets each containing a predetermined amount of the active ingredient, ranging from about

10 to 1000 mg, more preferably 10-200 mg per day per patient, even more preferably 20-200 mg per day per patient, in a pharmaceutically acceptable carrier. The compound can be formulated with or without pharmaceutically-acceptable carriers customarily used in the compounding of solid dosage forms. Preferably, the capsule or tablet is designed so that the active portion of the formulation is released at that point in the gastro-intestinal tract when bioavailability is maximized and pre-systemic degradation is minimized. Additional excipients can be included to facilitate absorption of the compound. Diluents, flavorings, low melting point waxes, vegetable oils, lubricants, suspending agents, tablet disintegrating agents, and binders can also be employed.

For parenteral administration, the compound is administered by either intravenous, subcutaneous or intramuscular injection, in compositions with pharmaceutically acceptable vehicles or carriers. For administration by injection, it is preferred to use the compound in solution in a sterile aqueous vehicle which may also contain other solutes such as buffers or preservatives as well as sufficient quantities of pharmaceutically acceptable salts or of glucose to make the solution isotonic. Subcutaneous injection is the preferred route of administration. Dosages are essentially the same as those set forth above for oral administration.

For local administration, the compound is preferably formulated to maximize the availability of the compound at the intended site of administration. Local administration of the compound at known or expected sites of entry or release of retrovirus into and from the body is particularly contemplated. For example, in consideration of the variety of mammalian sexual practices, topical administration of the compound to all orifices and interior or exterior genitalia is desirable. In addition, topical application to skin surfaces, and in particular to any

skin interruptions such as cuts, abrasions, lesions, blisters and the like, is believed useful in preventing the exchange of retrovirus from one host to another.

In one particularly useful embodiment, gloves containing the compounds formulated for local administration are prepared for those who come into contact with hosts or bodily fluids. Preferred dosages for local administration include those for which a local compound concentration of 1-10 ug/ml is achieved.

In the case of systemic administration, the specific dose is typically calculated according to the approximate body weight of the patient. On the other hand, for local administration, the specific dose is typically not a function of the patient's body weight. Other factors in determining the appropriate dosage can include the disease or condition to be treated or prevented, route of administration and the age, sex and medical condition of the patient. Generally, treatment is initiated with small dosages substantially less than the optimum dose of the compound. Thereafter, the dosage is increased by small increments until the optimum effect under the circumstances is reached.

In general, the compound is most desirably administered at a concentration level that will generally afford antivirally effective results without causing any harmful or deleterious side effects. It is desirable to maintain a blood level of the compound at a level sufficient to inhibit retrovirus infection of the host cell. This can be estimated by assaying the amount of compound that is effective in preventing retroviral infection of host cells, e.g., HIV into monocytes, in vitro, and then, using standard pharmacokinetic techniques, determining the amount of compound required to keep plasma level at the same inhibitory level, or up to 10-100 times more. In certain embodiments, the dosage and administration is designed to create a preselected concentration range of the compound in the patient's blood stream. Further refinement of the calculations necessary to determine the

appropriate dosage for treatment involving each of the above mentioned formulations is routinely made by those of ordinary skill in the art and is within the ambit of tasks routinely performed by them without undue experimentation, especially in light of the dosage information and assays disclosed herein. These dosages may be ascertained through use of the established assays for determining dosages utilized in conjunction with appropriate dose-response data.

Although the formulations disclosed hereinabove are effective and relatively safe medications for treating HIV infections, the possible concurrent administration of these formulations with antibacterial or other antiviral medications or agents to obtain beneficial results is not excluded. Such other antiviral medications or agents include soluble CD4, zidovudine, dideoxycytidine, phosphonoformate, ribavarin, antiviral interferons (e.g. alpha -interferon or interleukin-2) or aerosol pentamidine. Particularly useful antibacterial agents include those which target sexually-transmitted pathogens or the opportunistic pathogens often associated with retroviral infection.

It should be noted that the compound formulations described herein may be used for veterinary as well as human applications and that the term "patient" should not be construed in a limiting manner. In the case of veterinary applications, the dosage ranges should be the same as specified above.

It is also possible to utilize the nucleic acid sequences for SLPI themselves as therapeutic agents. For example, gene transfer methodologies can be employed to transfer a coding sequence for SLPI or an analog thereof to the patient where the gene can be replicated and expressed *in vivo*. Particularly useful gene therapy methods are discussed in the published international application

WO 93/00051, which is specifically incorporated herein by reference.

Compositions containing the compounds useful for storing or treating instruments, devices, or the like which contact hosts or bodily fluids are also within the scope of the invention. Nonlimiting examples of such instruments, devices , or the like include needles, speculums, scalpels, surgical clips, or other articles which might penetrate a host or contact bodily fluids. Preferably, the compositions neither adversely affect the activity of the compounds contained therein nor adversely react with the articles to be treated or stored. Such compositions can be prepared by methods known in the art particularly in light of the information contained herein.

The invention also includes a method for inhibiting retrovirus infection by blocking the function of a host cell enzyme, which enzyme function is necessary for retrovirus infection of the cell. As stated above, SLPI is a potent inhibitor of elastase, trypsin, cathepsin G, and chymotrypsin.

The host cell enzyme can be an elastase-like enzyme. The term "elastase-like enzyme" as used herein means a protease which cleaves at the carboxy-terminal side of amino acids with small to medium sized hydrophobic side chains such as leucine, isoleucine, valine, and alanine.

The host cell enzyme can also be a chymotrypsin-like enzyme. As used herein, the term "chymotrypsin-like enzyme" means a protease which cleaves at the carboxy-terminal side of amino acids with medium to large hydrophobic side chains, including for example, phenylalanine, tyrosine, trytophan, leucine, and isoleucine.

A trypsin-like enzyme can also be a host cell enzyme. The term "trypsin-like" enzyme as used herein means a protease which cleaves at the carboxy-terminal side of basic amino acids including, for example, lysine and arginine.

In addition, cathepsin G can also be a host cell enzyme.

The invention is exemplified by the following illustrative examples:

Example 1. Peripheral blood monocytes (PBM) were isolated from healthy donors by elutriation, plated in culture dishes, and incubated for several days. SLPI was mixed with HIV (Bal) and applied to PBM for one hour at 37°C. Cells were washed and incubated for additional time, with media changes and reverse transcriptase determinations on supernatants done every three days. We found that SLPI effectively blocks HIV replication at a concentration of 1 μg/ml (Figure 1). At concentrations $\leq$ 20 μg/ml, SLPI inhibition is diminished. The inhibitory effect is long lasting, with significant inhibition seen out to 18 days (Figure 2).

Example 2. PBM were plated and incubated as in Example 1. SLPI was applied to cells for about one hour, cells were then washed, and treated with HIV. Medium was changed and assays done as in Example 1. We found that SLPI was more effective at blocking HIV when cells were pretreated with SLPI than when cells were treated with a mix of SLPI and HIV.

Example 3. We have also demonstrated using essentially the same protocol as in Example 1, but substituting T-cells for monocytes, that SLPI is effective in inhibiting HIV replication in T-cells.

Example 4. A human T-lymphocytic cell line (H-9) was maintained in suspension culture in RPMI 1640 with 10% fetal calf serum (FCS) and 200 micrograms per liter gentamicin. SLPI was added to the culture medium at a final concentration of 100 micrograms per milliliter. After 24 hours, cells were washed, inoculated for four hours with HIV strain IIIB, washed again, and resuspended at a density of 500,000 cells per milliliter. Media was supplemented and maintained with SLPI at a final concentration of 100 micrograms per milliliter immediately after resuspension

(T=0) or 2 days after resuspension (T=2). Culture supernatant was collected and cultures were fed every 2 days. Supernatant collected 8 days after infection was assayed for reverse transcriptase activity by measuring uptake of tritiated thymidine onto a poly(rA)-oligo(dT) template.

As shown in Table 1, in SLPI pretreated cells, SLPI inhibited viral replication by approximately 62% and 54% when added immediately after infection and 2 days after infection, respectively.

TABLE 1

SLPI PRE-TREATED CELLS

	Negative Control	Positive Control	T = 0	T = 2
RT Activity (mean cpm)	955	86,205	32,594	39,554
Standard Deviation	± 330	± 9,676	± 7,220	± 8,737

Example 5. The experiment was performed as described in Example 4 except that 1000-fold concentrated HIV strain IIIB was incubated with 100 micrograms per milliliter SLPI for 6 hours on ice prior to inoculation. This HIV/SLPI mixture was diluted 1000-fold prior to the four hour inoculation.

As shown in Table 2, using SLPI pre-treated virus and cells, SLPI inhibited viral replication by approximately 64% and 26% when added immediately after infection and 2 days after infection, respectively.

TABLE 2

SLPI PRE-TREATED VIRUS AND CELLS

	Negative Control	Positive Control	T = 0	T = 2

RT Activity (mean cpm)	2,889	59,004	20,676	43,432
Standard Deviation	± 565	± 10,988	± 4,111	± 14,982

Example 6. The experiment was performed as in Example 5 except that cells were clean, i.e. not cultured with SLPI prior to inoculation. Using clean cells and SLPI pre-treated virus, SLPI inhibited viral replication by approximately 59% and 32% when added immediately after infection and 2 days after infection, respectively (Table 3).

TABLE 3

SLPI PRE-TREATED VIRUS

	Negative Control	Positive Control	T = 0	T = 2
RT Activity (mean cpm)	4,763	70,076	28,383	47,436
Standard Deviation	± 1,698	± 15,803	± 5,520	± 11,679

Example 7. The experiment was performed as in Examples 4-6 except that neither cells nor virus were exposed to SLPI prior to inoculation. Using clean cells and clean virus, SLPI inhibited viral replication by approximately 50% and 42% when added immediately after infection and 2 days after infection, respectively (Table 4). Table 5 shows the reverse transcriptase activity which was present in culture supernatant assayed 4, 6, and 8 days after infection.

TABLE 4

CLEAN CELLS AND VIRUS

	Negative Control	Positive Control	T = 0	T = 2
RT Activity (mean cpm)	531	79,356	38,969	46,004
Standard Deviation	± 186	± 17,497	± 7,700	± 8,492

TABLE 5

CLEAN CELLS AND VIRUS

	Negative	Positive	T = 0	T = 2
Day 4 (mean cpm)	435	1,556	797	1,287
Standard Deviation	± 85	± 300	± 222	± 204
Day 6 (mean cpm)	952	72,085	15,846	41,240
Standard Deviation	± 715	± 12,219	± 5,644	± 14,542
Day 8 (mean cpm)	1,519	13,853	7,617	11,946
Standard Deviation	± 475	± 3,458	± 3,031	± 2,889

Example 8. The effect of different SLPI muteins on viral replication was also investigated. Clean H-9 cells were incubated with clean virus for 4 hours as in Example 7. After washing, cells were resuspended at a density of 500,000 cells per milliliter in media containing 30 micrograms per milliliter SLPI or the SLPI muteins shown in Table 6. Culture supernatant was assayed for reverse transcriptase activity 8 days later (Table 6).

TABLE 6

	Negative Control	Positive Control	Wild Type	Gly 20	Gly 72	Val 72	Lys 72	Phe 72
RT Activity (mean cpm)	4,815	55,126	39,323	39,387	40,549	36,077	52,239	8,384
Standard Deviation	± 2,849	± 6,637	±10,933	±11,143	± 3,537	± 7,859	± 5,863	±1,924

Example 9. The experiment was performed as in Example 8 except that after inoculation, cells were resuspended in media containing 100 micrograms per milliliter SLPI or the Phe 72 mutein. Culture supernatant was assayed for reverse transcriptase activity 2, 4, 6, 8, and 10 days post-infection (Table 7). Tables

6 and 7 show that the effect of the Phe-72 mutein was particularly pronounced.

TABLE 7

	Negative	Positive	SLPI	PHE-72
Day 2 (mean cpm)		1,386	995	897
Standard Deviation		± 914	± 246	± 472
Day 4 (mean cpm)		1,356	1,087	1,380
Standard Deviation		± 370	± 414	± 442
Day 6 (mean cpm)	1,142	2,103	1,526	748
Standard Deviation	± 389	± 498	± 508	± 243
Day 8 (mean cpm)		77,931	25,241	3,491
Standard Deviation		± 9,779	± 8,399	± 1,086
Day 10 (mean cpm)		21,431	12,499	2,239
Standard Deviation		± 1,890	± 3,495	± 444

Example 10. To determine the effect of SLPI alone, H-9 cell proliferation was evaluated by thymidine incorporation assays using 200,000 H-9 cells cultured with 100 micrograms per milliliter SLPI and without SLPI. Cultures were pulsed with media containing 2.5 microcuries of tritiated thymidine at day 0, 1, and 2; incorporated counts were measured on day 1, 2, and 3. As shown in Table 8, SLPI is not toxic to these cells.

TABLE 8

THYMIDINE UPTAKE (mean cpm)

H-9 PROLIFERATION

	Day 1	Day 2	Day 3
Control (- SLPI)	20,860	67,401	53,326
Standard Deviation	± 581	± 2,529	± 3,783
+ SLPI 100 µg/ml	20,437	61,892	54,592
Standard Deviation	± 1,503	± 216	± 2,781

Example 11. We also investigated inhibition of viral production from chronically infected cells using the promonocytic cell line U1. Suspension cultures of U1 were maintained in RPMI with 10% FCS and 200 micrograms per liter gentamicin. Cells were harvested, washed, and suspended at a density of 2.5 million cells per milliliter. Suspended cells were cultured overnight in media containing 100 or 200 micrograms per milliliter SLPI or media alone. Virus was induced by addition of 13-phorbol-12-myristate acetate (PMA) to a final concentration of 1 micromolar. After 48 hours, cell culture supernatant was assayed for reverse transcriptase activity as in Examples 4-9. As shown in Table 9, SLPI significantly inhibited viral production from these chronically infected cells.

TABLE 9

	- PMA - SLPI	- PMA + SLPI (200 µg/ml)	+ PMA - SLPI	+ PMA + SLPI (200 µg/ml)	+ PMA + SLPI (100 µg/ml)
RT Activity (mean cpm)	1,052	994	5,052	2,864	2,648
Standard Deviation	± 352	± 447	±2,053	± 403	± 374

Example 12 SLPI was shown to directly bind U937 cells, which are derived from a human monocyte cell line, and HuT78 cells, which are derived from a human T cell line. SLPI was radiolabelled with I-125, having a specific activity of 125 Curies per millimole, using the Bolton Hunter reagent. The labeled protein had normal anti-elastase and anti-trypsin activity. Activity against cathepsin G and chymotrypsin was not determined. Specific binding of radiolabelled SLPI ("*SLPI") was determined by mixing increasing concentrations of *SLPI with cells (10^7/ml), in the absence and presence of cold SLPI using the procedure set forth in Dripps et al., J. Biol. Chem., Vol. 266, No. 16, pp. 10331-10336 (1991), specifically incorporated herein by reference. A Scatchard plot of the data, shown in Tables 10 and 11, gave an apparent K_D of 2nM for U937 cells and HuT78 cells. This shows that SLPI binds with high affinity to U937 cells and that the binding is physiologically relevant based on the concentration of SLPI in body fluids. Kramps, J.A. et al., AM REV RESPIR DIS, Vol. 129, pp. 959-963 (1984), specifically incorporated herein by reference.

TABLE 10-HuT78 CELLS

pM *SLPI	specific cpm	bound pM	bound per free	sites bound per cell
40	0	not done	not done	not done
80	30	0.75	0.0095	45
160	37	0.93	0.0058	56
320	101	2.53	0.0079	152
640	101	2.53	0.0039	152
1280	303	7.6	0.0059	458
2560	398	9.98	0.0039	601
5120	538	13.5	0.0026	813
10,240	1302	32.7	0.0032	1970
20,480	1210	30.3	0.0015	1825

TABLE 11-U937 CELLS

pM *SLPI	specific cpm	bound *SLPI(pM)	bound per free	sites bound per cell
40	380	9.5	0.311	572
80	543	13.6	0.205	819
160	1315	32.9	0.259	1,982
320	2621	65.7	0.258	3,957
640	4630	116.1	0.221	6,993
1280	8650	217	0.204	13,070
2560	13,500	339	0.153	20,418
5120	15,124	379	0.080	22,827
10,240	18,462	463	0.047	27,886
20,480	18,769	471	0.024	28,368

Example 13 U937 cells were induced by 13-phorbol-12-myristate acetate ("PMA"). Hanson et al., J. Biol. Chem., Vol. 265, pp. 1524-1530 (1990); and Welgus et al., J. Clin. Invest., Vol. 77, pp. 1675-1681 (1986), specifically incorporated herein by reference, show that PMA induction of U937 cells reduces cathepsin G and elastase activity.

U937 cells at an initial concentration of 10,000 cells per ml were placed in two flasks. PMA was added to one flask at a final concentration of 25ng per ml. After 48 hours, cells were harvested from both flasks. Using the method of Dripps et al., J. Biol. Chem., Vol. 266, No. 16, pp. 10331-10336 (1991), and labelling with I-125, having a specific activity of 91.5 Curries per millimole, the number of SLPI binding sites was determined. A Scatchard plot of the data in Tables 12 and 13 shows the number

of SLPI binding sites in the PMA induced cells was reduced by 70%. In this experiment, the K_d in non-induced cells was 1.1nM; The K_d in PMA-induced cells was 1.0 nM. The reduction in the number of SLPI binding sites in the PMA-induced cells is consistent with SLPI binding to elastase and cathepsin G on the cell surface.

TABLE 12 U937 CELLS-MINUS PMA

pM *SLPI	specific cpm	bound *SLPI (pM)	bound per free	sites bound per cell
80	400	37	0.860	2,229
320	1,077	101	0.461	6,083
640	2,213	207	0.478	12,468
1280	4,234	396	0.448	23,851
2560	6,340	593	0.301	35,716
5120	7,989	748	0.171	45,052
10,240	9,022	844	0.090	50,834
20,480	9,783	915	0.047	55,110

TABLE 13 U937 CELLS-PLUS PMA

pM *SLPI	specific cpm	bound (pM)	bound per free	sites bound per cell
80	339	32	0.67	1,909
320	698	65	0.255	3,915
640	1,103	103	0.192	6,204
1,280	1,391	130	0.113	7,830
2,560	2,302	215	0.092	12,949
5,120	1,442	135	0.027	8,131
10,240	2,933	274	0.028	16,503
20,480	2,725	255	0.0126	15,358

Example 14 The binding of SLPI to U937 and HuT78 cells was shown to be specific. *SLPI was competitively inhibited with cold SLPI to these intact cells using the procedure set forth in Dripps et al., J. Biol. Chem., Vol. 266, No. 30, pp. 20311-20315 (1991), specifically incorporated by reference. The percentage of *SLPI that was bound at various nanomolar concentrations of cold SLPI is set forth in Table 14. Specific binding was not inhibited by other basic proteins or other protease inhibitors at the concentrations listed in Table 15.

TABLE 14. COMPETITIVE INHIBITION OF *SLPI BINDING

SLPI(nM)	% *SLPI BOUND (U937)	% *SLPI BOUND (HuT78)
0.00	94	100
0.024	100	88
0.078	101	90
0.25	92	69
0.80	77	62
2.6	55	56
8.2	24	not done
26	12	37
84	3.5	9.3
270	0.00	0.00
860	0.00	8.7
2750	0.50	not done

TABLE 15 COMPETITION STUDY USING VARIOUS COMPOUNDS

COMPOUND	CONCENTRATION	COMMENTS
HPSTI	2.7μM	INHIBITS TRYPSIN
bFGF	2.7μM	STRONGLY BASIC PROTEIN
PMSF	10mM	INHIBITS TRYPSIN
BENZAMIDINE	10mM	INHIBITS TRYPSIN
APROTININ	10μM	INHIBITS TRYPSIN
LEUPEPTIN	10μM	INHIBITS TRYPSIN
ANTIPAIN	10μM	INHIBITS TRYPSIN
α-1-ANTITRYPSIN	10μM	INHIBITS TRYPSIN AND ELASTASE
PEPSTATIN A	10μM	INHIBITS PEPSIN

<u>Example 15</u> SLPI muteins were also shown to competitively inhibit SLPI binding to U937 cells and HuT78 cells using the procedure set forth in Example 14. (Dripps et al., <u>J. Biol. Chem.</u>, Vol. 266, No. 30, pp. 20311-20315 (1991)). Table 16 shows the affinities, as expressed by dissociation constant, K_d, of SLPI and of various SLPI muteins for U937 and for HuT78 cells, as estimated by the Cheng-Prusoff relationship. The Cheng-Prusoff relationship is set forth in Dripps et al., <u>J. Biol. Chem.</u>, Vol. 266, No. 30, pp. 20311-20315 (1991). Tables 17 and 18 show the percentage of radiolabelled SLPI bound at various concentrations of cold SLPI and of SLPI muteins to U937 and HuT78 cells, respectively.

TABLE 16

AFFINITIES OF SLPI AND SLPI MUTEINS FOR U937 AND HuT78 CELLS

MUTEIN	SLPI	GLY 72	GLY 20	LYS 72	PHE 72	VAL 72
K_d (nM) U937	2	400	3.5	2.5	2	30
K_d (nM) HuT78	2	200	13	200	3	9

TABLE 17 COMPETITION STUDY USING SLPI MUTEINS.

% *SLPI BOUND TO U937 CELLS AT VARIOUS CONCENTRATIONS OF (COMPETITORS)

nM COMPETITOR	%*SLPI BOUND (SLPI)	%*SLPI BOUND (GLY72)	%*SLPI BOUND (GLY20)	%*SLPI BOUND (LYS72)	%*SLPI BOUND (PHE72)	%*SLPI BOUND (VAL72)
0	109	131	130	117	94	95
0.024	111	116	110	116	not done	not done
0.078	111	118	117	113	not done	not done
0.25	106	120	110	103	not done	not done
0.80	78	116	91	86	77	90
2.6	46	115	not done	71	51	80
8.2	22	117	49	39	22	78
26	11	112	28	21	not done	not done
84	7	101	11	10	4	48
270	5	75	6	6	not done	not done
860	4	53	5	5	not done	not done
2750	4	28	5	4	5	8

TABLE 18 COMPETITION STUDY USING SLPI MUTEINS.
% *SLPI BOUND TO HuT78 CELLS AT VARIOUS CONCENTRATIONS OF (COMPETITORS)

nM COMPETITOR	%*SLPI BOUND (SLPI)	%*SLPI BOUND (LYS72)	%*SLPI BOUND (GLY72)	%*SLPI BOUND (GLY20)	%*SLPI BOUND (PHE72)	%*SLPI BOUND (VAL72)
0	100	100	100	100	100	100
0.80	79	105	108	109	69	93
2.6	76	118	105	91	90	96
8.2	57	116	85	103	57	81
84	27	86	110	50	36	43
2750	0	34	28	7	1	14

Example 16 SLPI and SLPI muteins were shown were shown to bind plasma membranes derived from U937 cells. Membranes were prepared as follows. Cells treated with a 10 mM NaCl solution were disrupted in a cell homogenizer. The plasma membrane fraction was enriched using a 41% sucrose step gradient. The membrane band was isolated and washed three times in phosphate buffered saline. Competitive binding, using the competitors set forth in Tables 19 and 20, to these membranes was performed as in Example 14 except that specific complexes were captured using the filtering procedure of Slack, J. et al., Biotechniques, Vol. 7, pp. 1132-1138 (1989), specifically incorporated herein by reference.

The affinities (K_d), as estimated by the Cheng-Prusoff relationship, for SLPI, SLPI muteins, gp120, alpha-1 protease inhibitor ("α-1-PI"), and aprotinin are shown in Table 19. The percentage of radiolabelled SLPI that was bound at various concentrations of cold SLPI or SLPI muteins is shown in Table 20. These results show that gp120 can compete with SLPI binding to its

target. α-1-PI and aprotinin can also compete but at reduced affinities. Since gp120 can be a substrate for the host cell enzyme, the ability of gp120 to compete can be hampered by its being cleaved.

TABLE 19 AFFINITIES OF SLPI AND SLPI MUTEINS FOR U937 MEMBRANES

COMPOUND	K_d (nM)
SLPI	2
PHE 72	1
GLY 72	5
GLY 20	5
LYS 72	5
VAL 72	10
gp120	400
α-1-PI	1,000
APROTININ	10,000

TABLE 20. CPM *SLPI BOUND AT VARIOUS CONCENTRATIONS OF COMPETITOR

COMPETITOR (nM)	0	0.800	2.56	8.20	83.9	268	2750
SLPI	18700	14753	10969	5709	2068	not done	1682
LYS72	18700	14194	12213	7615	3497	3235	5008
GLY72	18700	14716	11994	7206	3711	2840	1785
PHE72	18700	12419	8919	4257	1662	2585	1537
VAL72	18700	14023	11616	9238	3381	1737	923
GLY72	18700	14036	11337	7298	3944	2531	1433
GLY20	18700	14915	11390	7395	2486	1620	1501
bFGF	18700	14010	13480	14464	14967	14354	14710
LYSOZYME	18700	15190	14659	15012	15477	14884	15948

Example 17 The binding sites on U937 and HuT78 cells for the Phe72 mutein were characterized. Membranes from U937 and Hut78 cells, prepared as described in Example 16, were treated with 0.25% CHAPS detergent and centrifuged, dialyzed in 20mM potassium phosphate, pH5.5, 0.25% CHAPS, and chromatographed on monoS column, and eluted with a linear salt gradient. Fractions were analyzed by their ability to bind to immobilized the Phe72 mutein on a BIAcore instrument. The BIAcore analytical system is available from Pharmacia Biosensor AB, Uppsala, Sweden. See, Nature, Vol. 361, pp. 186-187 (1993), specifically incorporated herein by reference.

From solubilized U937 membrane monoS fractions, 4 peaks of binding activity to the Phe72 mutein were seen; peak 1 was from the monoS column flowthrough, peak 2 and 3 comigrated on monoS with purified human neutrophil elastase ("HNE") obtained from

Calbiochem, San Diego, California. Peak 4 appears to be cathepsin G based on comparison with the monoS profile of cathepsin G appearing in Maison, C.M. et al., Journal of Immunology, Vol. 147, pp. 921-926 (1991), specifically incorporated herein by reference.

From HuT78 membranes, 1 peak of binding activity to the Phe72 mutein was seen. This peak coeluted on monoS with the second peak of activity from U937 membranes.

Using reducing SDS-PAGE, Western blot analysis of these active peaks showed that peaks 2 and 3 from U937 and the single peak from Hut78 contain a 30kDa protein that reacts with an anti-elastase polyclonal antibody obtained from Calbiochem.

Affinity chromatography of CHAPS solubilized U937 membranes on a Phe72 SLPI mutein affinity column, prepared using NHS-activated Superose, PC 3.2/2 obtained from Pharmacia Biotech Inc., Piscataway, NJ, resulted in isolation of a doublet of proteins with the same mobility on reducing SDS-PAGE as active peaks 2 and 3. NHS-activated Superose, PC 3.2/2 is described at page 8 in the Pharmacia Biotech Inc. Biotechnology Products Catalog 1994.

Example 18 SLPI and SLPI muteins were shown to inhibit proteolysis of the HIV-1 envelope protein, gp120, by human neutrophil elastase ("HNE"). As stated above, gp120 specifically binds to the CD4 receptor on T lymphocytes and on monocytes and macrophages.

Inhibition of HNE proteolytic cleavage of gp120 was shown as follows. The gp120 (400nM), obtained from American Bio-Technologies, Cambridge, Massachusetts, was incubated with increasing HNE (0.1 to 100nM) concentrations. Incubation at 37° for 30 min, with 10nM HNE yielded primarily 2 bands on Western blot, using reducing SDS-PAGE and probing with an anti-gp120 polyclonal antibody from American Bio-Technologies. The bands had an approximate molecular weight of 50kDa and 70kDa. This limited

HNE proteolysis was inhibited by an anti-V3 loop monoclonal antibody (0.2 to 4 μM), also obtained from American Bio-Technologies, and by SLPI and SLPI muteins Phe72, Gly72, and Lys72 (0.1 to 100 nM). The IC50 of Lys72 appeared to be higher than that of SLPI or the other SLPI muteins. This is consistent with the observation that the Lys72 mutein is less effective in inhibiting HIV replication than wild type SLPI, the Phe72, and Gly72 muteins. (See Table 6, Example 8, above).

Purified soluble CD4 (400nM), obtained from American Bio-Technologies, did not inhibit this cleavage.

gp120 (400nM) was also incubated at 37° for 30 min, with 10nM cathepsin G and yielded different bands. This suggests that cathepsin G does not cleave gp120 in the V3 loop.

Example 19 Radiolabelled SLPI ("*SLPI") was shown to bind to elutriated human peripheral blood monocytes ("PBM"). SLPI was radiolabelled using $Na^{125}I$, obtained from ICN, 2727 Campus Dr., Irving, CA 92715, and Iodogen, obtained from Pierce, Rockford, IL, according to the manufacturers' directions. Radiolabelling yielded 1.3 microcuries per microgram of SLPI. PBM (20×10^6) in 1 ml Dulbecco's-modified Eagle's medium ("DMEM") were incubated with 0.6nM to 13.2 nM *SLPI for 1 hour at 37° C. Cells were then pelleted and washed 3 times with phosphate buffered saline ("PBS"). Cell pellets were resuspended in Laemmli sample buffer and the samples applied to a 14% polyacrylamide gels under reducing conditions. Gels were analyzed using a phosphor imager to detect ^{125}I emission.

Alternatively, PBM (12×10^6) in 1 ml DMEM were incubated for 30 minutes at 4° C with increasing amounts of *SLPI. Cells were layered onto a 20% sucrose cushion and centrifuged 4 times in an Eppendorf microfuge. Cell pellets were removed and analyzed for gamma emission. Nonspecific binding, determined by including a

1000-fold excess of unlabelled SLPI was subtracted from total binding to give specific binding.

*SLPI binding was dose dependent and saturable at approximately 0.5 micrograms/ml *SLPI. The *SLPI concentration required for half-maximal binding to PBM was determined to be approximately 10nM. This value (10nM) is similar to the K_d of *SLPI for U937 cells (see Examples 12 and 13).

In addition, *SLPI was crosslinked to PBM. PBM (4×10^6) in 200 μl DMEM were incubated at 37° C for 1 hour with 1.5 μM *SLPI. Cells were spun down and washed 3 times at room temperature with PBS and resuspended in 100 μl PBS. Either 0.5mM DSS or 0.5mM BS^3, were added for 30 minutes at room temperature. DSS is an organic crosslinking agent; BS^3 is a water soluble crosslinking agent. Both cross-linking agents were obtained from Pierce, Rockford, IL, After incubation with the crosslinking agent, the cells were spun down and resuspended in Laemmli sample buffer and analyzed on a 4-15% gradient polyacrylamide gel under reducing conditions. Gels were dried and emission analyzed using a phosphor imager. Control samples for cross-linking consisted of *SLPI and cross-linking agent without monocytes.

In gels of crosslinked monocytes, using either cross-linking agent, one major crosslinked band appeared. This band was not seen in the control samples. Under reducing and nonreducing conditions, the molecular weight of the band was approximately 30kDa. This molecular weight is consistent with the binding of SLPI (12kDa) to elastase (25kDa).

Example 20 Using antibody staining, elastase was shown to be present on the surface of human monocytes. Purified rabbit polyclonal antibody to human neutrophil elastase ("Anti-HNE") was obtained from Calbiochem, LaJolla, CA. Anti-HNE was conjugated to fluorescein isothiocyanate ("FITC") using the procedure set forth in Current Protocols in Immunology, 5.3.2 - 5.3.3, Coligan et al.

eds. (1993), specifically incorporated herein by reference. Elutriated human monocytes were stained with either FITC-conjugated IgG (control antibody), FITC-conjugated anti-HNE ("FITC-anti-HNE"), or FITC-conjugated antibody against the monocyte marker, CD14. Control IgG antibody and FITC-conjugated anti-CD14 antibody were obtained from Becton Dickinson, San Jose, CA.

The cells (0.5×10^6 in 50μl buffer) stained positive in a dose-related manner using 1μg/ml - 50μg/ml anti-HNE. Staining by the anti-CD14 antibody confirmed that the cells were monocytes. No staining was seen with the control IgG.

Example 21 In cross-linking experiments, SLPI did not directly interact with HIV-1. In separate experiments, approximately equal amounts of HIV-1 strains IIIB or BAL were incubated with 1μM radiolabelled SLPI (*SLPI) in 200μl DMEM for 1 hour at 37° C. After 1 hour, crosslinking reagent DSS (see Example 19) was added and incubation continued for 30 minutes at room temperature. Incubation was stopped by addition of Laemmli sample buffer. No attempt was made to remove free *SLPI from virus. Samples were applied to a 12% polyacrylamide gel run under reducing conditions. In a parallel sample, 2μM *SLPI was crosslinked in the absence of virus. The volume of the parallel sample that was loaded on the gel was 5 times larger than the volume of the virus-containing samples. Gels were dried and analyzed using a phosphor imager to detect ^{125}I emission. No crosslinked products were detected in the viral samples. Only free *SLPI was observed. In the parallel sample, multiple crosslinked bands (SLPI crosslinked to itself) were seen.

Example 22 SLPI was shown to neither activate nor inhibit monocyte activation by lipopolysaccharide ("LPS"). Elutriated human monocytes (5×10^6/ml in DMEM with 10% human serum) were incubated overnight at 37° C, in the presence of 5% CO_2 with:

(1) medium alone (control);

(2) 1μg/ml LPS;

(3) SLPI at 1μg/ml, 10μg/ml, or 100μg/ml; or

(4) 1μg/ml LPS plus SLPI at 10μg/ml or 100μg/ml.

After overnight incubation, the cells from each of the seven groups were divided into 4 aliquots and stained using FITC-conjugated antibodies against three cell surface antigens and an FITC-conjugated IgG as a control. The three surface antigens probed were: (1) interleukin 2 receptor (IL-2R); (2) CD4; and 3) CD14.

The stained cells were analyzed by fluorescence-activated cell sorting ("FACS") and the results are shown in Table 21. The percent of cells labelled was calculated by percent labelled with specific antibody minus percent labelled with non-specific control antibody.

Control cells stained negative for IL-2R, which is a marker for cell activation. Control cells stained slightly positive for CD4 and positive for CD14, a monocyte marker.

Cells incubated overnight with LPS were activated, as demonstrated by positive IL-2R staining. In the LPS-activated cells, the CD4 staining disappeared and CD14 remained positive.

Cells incubated with SLPI in the absence of LPS stained negative for IL-2R. Thus, SLPI did not activate the monocytes. Cells incubated with SLPI and LPS, however, stained positive for IL-2R. Thus, SLPI did not interfere with LPS activation of monocytes.

TABLE 21

FACS ANALYSIS OF SLPI EFFECT ON MONOCYTE CELL SURFACE ANTIGENS

CELL TREATMENT	IL-2R	CD4	CD14
CONTROL	neg.	28.3%	93%
LPS(1μg)	124%	neg.	131%
SLPI(1μg)	neg.	30.5%	87.9%
SLPI(10μg)	neg.	27.4%	115%
SLPI(100μg)	neg.	32.2%	104%
LPS(1μg) +SLPI(10μg)	132.2%	neg.	104.5%
LPS(1μg) +SLPI(100μg)	131.2%	neg.	137%

The foregoing description of the invention is exemplary for purposes of illustration and explanation. It should be understood that various modifications can be made without departing from the spirit and scope of the invention. Accordingly, the following claims are intended to be interpreted to embrace all such modifications.